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(54) Title: PAN-SPECIFIC ANTI-ALLERGY THERAPY

(57) Abstract

Compositions are administered to block IgE binding to receptors and ultimately displace native IgE from mast cells and related cell types, to prevent the activation of these cells during an allergic response. The compositions consist of a pharmaceutically acceptable carrier for systemic or local administration and an amount of compound binding specifically to the FcεRI IgE binding sites, and more preferably, FcεRI and FcεRII IgE binding sites, to prevent activation and degranulation of mast cells in response to exposure to allergens. The compounds can consist of IgE molecules and fragments and modifications thereof, such as IgE fragments, humanized or single chain IgE antibodies or fragments thereof, IgE with a modified Fab, non-cross-linkable IgE, or peptidomimetics which bind to the same site on the receptor as the IgE, jointly referred to herein as "IgE fragments" unless otherwise stated.

PAN-SPECIFIC ANTI-ALLERGY THERAPY

Background of the Invention

The symptoms of allergy in humans and animals are primarily

5   attributable to the release of histamine and a large variety of other bioactive

compounds from mast cells and related cell types. The mast cell contains

numerous secretary granules in which these substances are stored at

extremely high concentrations. Activation of the mast cell results in the

fusion of these granules with the cell surface membrane, leading to the

10   exocytosis of the granule contents and the concomitant induction of allergic

symptoms. The plasma membrane of these cells are endowed with receptors

for the Fc portion of the IgE (FcεRI). This receptor binds circulating IgE

with very high affinity and retains it at the mast cell surface for extended

periods of time. Activation is accomplished through the binding of an

15   allergen simultaneously to more than one polyvalent molecule of FcεRI-

bound IgE. This "cross linking" of at least two surface-bound IgE molecules

brings FcεRI proteins into close association with one another in the plane of

the mast cell plasma membrane. Kinases associated with these receptors

become activated as a result of this proximity, initiating the second

20   messenger cascade which results in cell degranulation.

At least one other class of receptors can bind to the Fc portion of IgE.

The low affinity receptor for IgE, FcεRII (also known as CD23) is expressed

on mast cells and related cell types, B cells, and subsets of antigen presenting

cells. It has been suggested that occupancy of FcεRII negatively regulates

25   IgE synthesis.

It is an object of the present invention to provide a means and method

of preventing activation and degranulation of mast cells and related cell types

in response to exposure to allergens.

Summary of the Invention

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Compositions are administered to block IgE binding to cell surface

receptors and ultimately displace native IgE from mast cells and related cell

types to prevent the activation of these cells during an allergic response and to reduce native IgE synthesis. The compositions consist of a pharmaceutically acceptable carrier for systemic or local administration and an amount of compound binding specifically to the FcεRI IgE binding sites,

5　and more preferably, FcεRI and FcεRII IgE binding sites, to prevent activation and degranulation of mast cells in response to exposure to allergens. The compounds can consist of IgE molecules and fragments and modifications thereof, such as IgE fragments, humanized or single chain IgE antibodies or fragments thereof, IgE with a modified Fab, non-crosslinkable

10　IgE, or peptidomimetics which bind to the same site on the receptor as the IgE, jointly referred to herein as "IgE fragments" unless otherwise stated.

Detailed Description of the Disclosure

As used herein, the term "mast cells" includes all cells expressing on

15　their surface FcεRI, including mast cells, basophils, and related cell types.

I.　　　**Compounds Specifically Binding to Mast Cell IgE Receptors.**

IgE Fragments which bind to IgE receptors

Allergen-induced release of mast cell granule contents can be prevented, or minimized, if the FcεRI IgE binding sites in the mast cell

20　plasma membrane are occupied with an analogue of IgE which is unable to bind antigen and thus is incapable of initiating receptor cross-linking. The domain of the IgE protein which binds to its receptor is termed the Fc portion. This component of the IgE molecule does not contain any of the variable regions which contribute to the formation of high affinity antigen

25　binding sites. When the Fc portion of IgE (or selected fragments of the Fc portion) are prepared by enzymatic cleavage or recombinant techniques, the resultant polypeptides bind with high affinity to mast cells. IgE Fc fragments can effectively block the binding of antigen-specific polyvalent native IgE to mast cell FcεRI. Consequently, Fc fragments of the IgE

30　molecule can prevent activation of mast cells by any antigen.

It has been suggested that occupancy of FcεRII negatively regulates IgE synthesis (Yu, et al. Nature 369: 753-756, 1994). Consequently,

pharmaceutical administration of IgE fragments is expected to both inhibit binding of native IgE to mast cells and to reduce the secretion of native IgE from patient B cells. To achieve this desirable outcome, it is necessary to administer an IgE fragment which retains the ability to interact with both

5   FcεRI and FcεRII.

       Preparation of Recombinant IgE Fc and Modifications to
       Increase Efficacy

       Extensive studies over the previous decade employing proteolysis,

10   site-specific antibodies, chimeric immunoglobulins and recombinant IgE fragments have demonstrated that the Fc portion of the IgE molecule, which is composed of three domains denoted Cε2, Cε3 and Cε4, binds to both FcεRI and FcεRII (Perez-Montfort, R. and H. Metzger. Molec. Immunol. 19:1113-1125, 1982; Rousseaux-Prevost, et al. Molec. Immunol. 24: 187-

15   196, 1987; Burt, et al. Molec. Immunol. 24: 379-389, 1987; Baniyash, et al. Molec. Immunol. 25:705-711, 1988; Weetall, et al. J. Immunol. 145: 3849-3854, 1990; Keegan, et al. Molec. Immunol. 28: 1149-1154, 1991; Moscoso del Prado, et al. Molec. Immunol. 28: 839-844, 1991; Nissim, et al. EMBO J. 10:101-109, 1991; Nissim, A. and Z. Eshhar. Molec. Immunol. 29:1065-

20   1072, 1992; Nissim, et al. J. Immunol. 150: 1365-1374, 1993; Helm, et al., J. Biol. Chem. 271: 7494-7500, 1996; Keown, Eur. Biophys J. 25: 471-476, 1997). The Fc polypeptide can block the binding of IgE to mast cells or basophils in vitro and can block the Prausnitz-Kustner passive sensitization reaction when injected subcutaneously in human subjects (Kenten, et al.

25   Proc. Nat. Acad. Sci. 81:2955-2959, 1984; Coleman, et al. Eur. J.Immunol. 15:966-969, 1985; Geha, et al. Nature. 315:577-578, 1985). The mapping studies demonstrate that the Cε3 region appears to be necessary and sufficient to confer high affinity binding to FcεRI. In contrast, specific association of IgE with FcεRII requires contributions from both the Cε3 and

30   Cε4 domains (Keegan, Molec. Immunol. 28: 1149-1154; 1991; Helm, et al. J. Biol. Chem. 271: 7494-7500, 1996; Vercelli, et al. Nature 338:649-651, 1989). Although a 76 amino acid fragment comprising portions of the Cε2 and Cε3 domains binds to the FcεRI and can inhibit the binding of native IgE

in vivo (Helm, et al. Proc. Nat. Acad. Sci. 86:9465-9469, 1989) and *in vitro* (Helm, et al. Nature 331: 180-183, 1988), the affinity of this molecule for the receptors is approximately 1/10 that of the entire Fc region.

Detailed analysis of the affinities of IgE fragments for FcεRI reveal

5   that the highest affinity is observed with the entire Fc region, which stretches from amino acids 226-547. The on and off rate constants and hence the equilibrium binding constant observed with this piece are essentially identical to the same parameters measured for native IgE (Helm, et al. J. Biol. Chem. 271: 7494-7500, 1996; Keown, et al. Eur. Biophys J. 25: 471-

10   476. 1997). Recombinant IgE fragments can be prepared by expression in *E. coli* (Kenten, et al. Proc. Nat. Acad. Sci. 81:2955-2959, 1984; Coleman, et al. Eur. J.Immunol. 15:966-969, 1985; Ishizaka, et al. Proc. Nat. Acad Sci, 83:8323-8327, 1986; Kurokawa, et al. Nucleic Acids Res. 11:3077-3085, 1983), yeast, insect cells (using a baculovirus system) or in transfected

15   mammalian cells (Ikeyama, Molec. Immunol. 24: 1039-1046, 1987). The protein forms large intracellular inclusion bodies when synthesized in *E. coli*. Following extraction from these inclusion bodies it can be dimerized by oxidative formation of a critical disulfide bond and gains full biological activity (Kenten, et al., 1984; Coleman, et al., 1985). IgE and IgE fragments

20   synthesized in *E. coli* is not glycosylated. Recent evidence indicates that synthesis in mammalian cells of an IgE Fc whose glycosylation sites have been eradicated by site-directed mutagenesis produces a molecule whose affinity for FcεRI is similar to that of native IgE (Young, et al. Protein Eng. 8:193-199, 1995). It would appear, therefore, that lack of glycosylation does

25   not disrupt the FcεRI-binding domain of IgE Fc and that material prepared either in mammalian cells or in *E. coli* should manifest similar biological activities. Furthermore, the non-glycosylated IgE Fc domain exhibits higher affinity binding to the low affinity FcεRII receptor than its fully glycosylated counterpart (Young, et al. Protein Eng. 8:193-199, 1995).

30   In a preferred embodiment, a cDNA sequence encoding amino acids 226-547 of the human IgE protein which corresponds to the portion of the IgE molecule then is essentially identical to native IgE with respect to its

affinity for both the FcεRI and FcεRII receptors (Helm, et al. J. Biol. Chem.
271: 7494-7500, 1996). Glycosylation sites at Asn 265 and Asn 371 can be
removed by site-directed mutagenesis so as to increase the molecule's
affinity for FcεRII (Young, et al. Protein Eng. 8:193-199, 1995). Lack of
5   glycosylation should also increase the serum half-life of the circulating
molecule, since it will not be a substrate for binding to the asialoglycoprotein
receptor (ASGPR), and thus will not be subject to the hepatic clearance and
degradation which binding to ASGPR initiates. A potential susceptibility
site for cleavage by the serum protease thrombin has been noted in the
10   sequence of the IgE Fc molecule (Kamiya, Human Antibodies and
Hybridomas 7:42-47, 1996). This site can be altered by site-directed
mutagenesis to ensure that the Fc molecule is not a substrate for thrombin-
mediated degradation. By preventing thrombin cleavage and ASGPR-
mediated clearance, it should be possible to attain higher levels of circulating
15   IgE fragments for longer periods of time than would be possible with the
native molecule. The resultant increase in the serum concentration of IgE
fragments will favor the binding of this molecule to the surfaces of patient
mast cells and will thus speed the displacement of native IgE required for its
therapeutic effects.
20   It is critically important that the IgE fragments described herein not
induce any immune reaction in the patients who receive it. Initiation of a
humoral immune response to this molecule would result in the production of
polyvalent antibodies which could cross-link the fragments bound to the
FcεRI receptors on mast cells surfaces. This cross-linking could, in turn,
25   activate the FcεRI signal cascade and lead to undesirable and potentially
catastrophic mast cell degranulation. All of the recombinant IgE Fc
fragments described to date have been prepared as fusion proteins.
Consequently, they retain protein sequences derived from the fusion
construct or from linkers which are not native to the IgE molecule. These
30   sequences are very likely to be immunogenic. Furthermore, the
incorporation of N-formyl-methionine at the N-terminus of bacterially
synthesized proteins increases the likelihood that IgE fragments generated

through bacterial expression will induce an immune response unless post-synthetic modifications are effected. It is unlikely, therefore, that any of the IgE Fc constructs described to date would possess any clinical utility.

These molecules must therefore be designed, or modified, so as to
5   ensure that the sequences described in the preceding paragraph are easily removable to avoid the problems posed by the potential immunogenicity of non-IgE derived sequences. For example, for expression in mammalian, insect or yeast cells, a DNA construct could be employed in which the nucleotide sequence encoding the leader peptide and N-terminal 10 amino
10 acids of rat preprolactin are fused to the sequence corresponding to amino acids 226-547 of IgE Fc. Interposed between the leader peptide sequence and the Fc coding sequence is a sequence encoding a His$_6$ tag followed by a Factor Xa cleavage site. The Fc coding sequence will be inserted immediately 3' to the sequence encoding the Factor Xa cleavage site.
15 The protein encoded by this cDNA construct will be translated in association with the rough endoplasmic reticulum (RER) and will be co-translationally transported across the RER membrane with concomitant cleavage of the leader peptide. The protein will pursue the secretory pathway and can be released constitutively from the cells. Metal ion
20 chromatography can be used to recover the secreted His-tagged protein from the culture media. Cleavage with Factor Xa will generate a protein whose N-terminal amino acid residue corresponds to amino acid 226 of the IgE Fc protein sequence. Cleaved protein will be purified by gel filtration chromatography.
25 A similar approach can be taken for bacterial expression. A methionine start codon will follow the promoter sequence, after which will be inserted the His$_6$ tag and the Factor Xa cleavage site. Bacterially synthesized protein will be recovered from the inclusion bodies, purified by metal ion chromatography, cleaved by Factor Xa and dimerized through
30 oxidation. Once again, the N-terminal residue will correspond to amino acid 226 of the IgE Fc sequence. Intact dimer will be prepared by gel filtration chromatography. The vectors employed to drive synthesis in mammalian,

insect or yeast cells or in bacteria will incorporate promoters designed to maximize exogenous protein expression.

Humanization of Antibodies

The IgE used to prepare the analogs can be human or animal, and will
5   typically be animal if monoclonal antibodies are used as a source. Since the methods for immunizing animals yield antibody which is not of human origin, the antibodies could elicit an adverse effect if administered to humans. This is also true if the antibodies are to be administered to any other species which is different from the species of origin of the antibodies.
10   As used herein, "humanization" refers to modifying the species-specific region of the antibody to be homologous to the species to be treated. Methods for "humanizing" antibodies, or generating less immunogenic fragments of non-human antibodies, are well known. A humanized antibody is one in which only the antigen-recognized sites, or complementarity-
15   determining hypervariable regions (CDRs) are of non-human origin, whereas all framework regions (FR) of variable domains are products of human genes. These "humanized" antibodies present a less xenografic rejection stimulus when introduced to a human recipient.

To accomplish humanization of a selected mouse monoclonal
20   antibody, the CDR grafting method described by Daugherty, *et al.*, Nucl. Acids Res., 19:2471-2476 (1991) may be used. Briefly, the variable region DNA of a selected animal recombinant anti-idiotypic ScFv is sequenced by the method of Clackson, T., *et al.*, Nature, 352:624-688, 1991. Using this sequence, animal CDRs are distinguished from animal framework regions
25   (FR) based on locations of the CDRs in known sequences of animal variable genes. Kabat, H.A., *et al.*, Sequences of Proteins of Immunological Interest, 4th Ed. (U.S. Dept. Health and Human Services, Bethesda, MD, 1987). Once the animal CDRs and FR are identified, the CDRs are grafted onto human heavy chain variable region framework by the use of synthetic
30   oligonucleotides and polymerase chain reaction (PCR) recombination. Codons for the animal heavy chain CDRs, as well as the available human heavy chain variable region framework, are built in four (each 100 bases

long) oligonucleotides. Using PCR, a grafted DNA sequence of 400 bases is formed that encodes for the recombinant animal CDR/human heavy chain FR protection.

The immunogenic stimulus presented by the monoclonal antibodies

5   so produced may be further decreased by the use of Pharmacia's (Pharmacia LKB Biotechnology, Sweden) "Recombinant Phage Antibody System" (RPAS), which generates a single-chain Fv fragment (ScFv) which incorporates the complete antigen-binding domain of the antibody. In the RPAS, antibody variable heavy and light chain genes are separately

10   amplified from the hybridoma mRNA and cloned into an expression vector. The heavy and light chain domains are co-expressed on the same polypeptide chain after joining with a short linker DNA which codes for a flexible peptide. This assembly generates a single-chain Fv fragment (ScFv) which incorporates the complete antigen-binding domain of the antibody.

15   Compared to the intact monoclonal antibody, the recombinant ScFv includes a considerably lower number of epitopes, and thereby presents a much weaker immunogenic stimulus when injected into humans.

Compounds Identified by Combinatorial Chemistry

It may be preferable to utilize non-peptide compounds to block

20   binding of IgE to the mast cell receptors. Molecules with a given function, for example, binding, can be selected for from a complex mixture of random molecules in what has been referred to as "in vitro genetics" (Szostak, TIBS 19:89, 1992) or combinatorial chemistry. One synthesizes a large pool of molecules bearing random and defined sequences and subjects that complex

25   mixture, for example, approximately 10^{15} individual sequences in 100 μg of a 100 nucleotide RNA or DNA, to some selection and enrichment process. For example, by repeated cycles of affinity chromatography and PCR amplification of the molecules bound to the ligand on the column, Ellington and Szostak (1990) estimated that 1 in 10^{10} RNA molecules folded in such a

30   way as to bind a given ligand. DNA molecules with binding behavior have also been isolated (Ellington and Szostak, 1992; Bock et al, 1992).

Using methodology well known to those of skill in the art, in

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combination with various combinatorial libraries, one can isolate and
characterize those compounds which bind to or interact with the desired
target. The relative binding affinity of these compounds can be compared
and optimum compounds identified using competitive binding studies which
are well known to those of skill in the art.

For example, the mast cell receptor(s), or relevant portions thereof,
can be bound to a solid support, and interacted with various combinatorial
libraries. Those molecules which do not bind these molecules at all are
removed immediately by elution with a suitable solvent. Those molecules
which bind to inactive portions of the receptor(s) can be removed by
competitive binding with an excess of a chimeric peptide with the inactive
portions represented by human sequences, or sequences from the desired
species, and the active portion represented by the sequence from another
species. Those compounds which bind to the receptor(s) will remain bound
to the solid support, whereas unbound compounds will be removed from the
column. Finally, those compounds still bound to the column can be
removed, for example, by competitive binding. Following removal, these
compounds can be identified and their relative binding affinity compared as
described above.

Rational Drug Design

Drugs with the ability to mimic the function of the portion of the IgE
which binds to the mast cell receptors can be identified using rational drug
design. The compounds preferably include the surface active functional
groups of the IgE, or substantially similar groups, in the same or
substantially similar orientation, so that the compounds possess the same or
similar biological activity. The surface active functional groups in the IgE
possess a certain orientation when they are in their active conformations, in
part due to their secondary or tertiary structure. Rational drug design
involves both the identification and chemical modification of suitable
compounds which mimic the function of the parent molecules.

Compounds that mimic the conformation and desirable features of a
particular peptide, e.g., an oligopeptide, but that avoid undesirable features,

e.g., flexibility (loss of conformation) and metabolic degradation, are known as "peptidomimetics". Peptidomimetics that have physical conformations which mimic the three dimensional structure of amino acids 226-547 of the human IgE protein, in particular, which have surface active groups as present

5   in this portion of the IgE, or peptidomimetics that have physical conformations which mimic the three dimensional structure of amino acids amino acids 226-547 of the human IgE protein can be used to make the pharmaceutical compositions described herein.

The physical conformation of the peptidomimetics are determined, in

10   part, by their primary, secondary and tertiary structure. The primary structure of a peptide is defined by the number and precise sequence of amino acids in the IgE. The secondary structure is defined by the extent to which the polypeptide chains possess any helical or other stable structure. The tertiary structure is defined by the tendency for the polypeptides to

15   undergo extensive coiling or folding to produce a complex, somewhat rigid three-dimensional structure.

Computer modeling technology allows visualization of the three-dimensional atomic structure of a selected molecule and the rational design of new compounds which will mimic the molecule or which will interact

20   with the molecule. The three-dimensional structure can be determined based on data from x-ray crystallographic analyses and/or NMR imaging of the selected molecule, or from *ab initio* techniques based solely or in part on the primary structure, as described, for example, in U.S. Patent No. 5,612,895 to Balaji et al. The computer graphics systems enable one to predict how a new

25   compound will link to the target molecule and allow experimental manipulation of the structures of the compound and target molecule to perfect binding specificity.

Many databases and computer software programs are known that can be used to design drugs. For example, see Ghoshal et al., "Computer Aids in

30   Drug Design - Highlights" (1996) Pol. J. Pharmacol. 48(4), 359-377; Wendoloski et al., "Biophysical Tools for Structure-Based Drug Design" (1993) Pharmacol. Ther. 60(2), 169-183; Lybrand, "Ligand-Protein Docking

and Rational Drug Design" (1995) Curr. Opin. Struct. Biol. 5(2), 224-228;

Kleinberg and Wanke, "New Approaches and Technologies in Drug Design

and Discovery" (1995) Am. J. Health Syst. Pharm. 52(12), 1323-1336;

Kubinyi, "Strategies and Recent Technologies in Drug Discovery" (1995)

5   Pharmazie 50(10), 647-662; Archakov et al., (1996) Vestn. Ross. Akad.

Med. Nauk. 1, 60-63; Taylor and Smith, "The Word Wide Web as a

Graphical User Interface to Program Macros for Molecular Graphics,

Molecular Modeling, and Structure-Based Drug Design" (1996) J. Mol.

Graph. 14(5), 291-296; Huang et al., "Development of a Common 3D

10   Pharmacophore for Delta-Opioid Recognition From Peptides and Non-

Peptides Using a Novel Computer Program" (1997) J. Comput. Aided Mol.

Des. 11(1), 21-78; and Li et al., "A computer Screening Approach to

Immunoglobulin Superfamily Structures and Interactions: Discovery of

Small Non-Peptidic CD4 Inhibitors and Novel Immunotherapeutics (1997)

15   Proc. Natl. Acad. Sci. USA 94(1), 73-78.

Data bases including constrained metabolically stable non-peptide

moieties may be used to search for and to suggest suitable IgE analogs.

Searches can be performed using a three dimensional data base for non-

peptide (organic) structures (e.g., non-peptide analogs, and/or dipeptide

20   analogs) having three dimensional similarity to the known structure of the

active regions of these molecules. See, e.g., the Cambridge Crystal Structure

Data Base, Crystallographic Data Center, Lensfield Road, Cambridge, CB2

1EW, England; and Allen, F. H., et al., Acta Crystallogr., B35: 2331-2339

(1979). Alternatively, three dimensional structures generated by other means

25   such as molecular mechanics can be consulted. See., e.g., Burkert, et al.,

Molecular Mechanics, American Chemical Society, Washington, D.C.

(1982); and Weiner, et al., J. Am. Chem. Soc., 106(3): 765-84 (Eng.) (1984).

Search algorithms for three dimensional data base comparisons are

available in the literature. See, e.g., Cooper, et al., J. Comput.-Aided Mol.

30   Design, 3: 253-259 (1989) and references cited therein; Brent, et al., J.

Comput.-Aided Mol. Design, 2: 311-310 (1988) and references cited therein.

Commercial software for such searches is also available from vendors such

as Day Light Information Systems, Inc., Irvine, Calif. 92714, and Molecular

Design Limited, 2132 Faralton Drive, San Leandro, Calif. 94577. The

searching is done in a systematic fashion by simulating or synthesizing

analogs having a substitute moiety at every residue level. Preferably, care is

5   taken that replacement of portions of the backbone does not disturb the

tertiary structure and that the side chain substitutions are compatible to retain

the IgE/receptor interactions. Using the information regarding bond angles

and spatial geometry of the critical amino acids, one can use computer

programs as described herein to develop peptidomimetics.

10   Chemical Modifications

            Chemically modified analogs of the active portion of the IgE

fragment can also be identified using the techniques described above.

Peptidomimetics can be modified to increase bioavailability. Preferably, the

compounds are structurally constrained such that the surface active groups

15   are oriented in the active conformation. The compounds can further include

chemical modifications that minimize the metabolic degradation of the

compounds once they are administered. See, for example, Spatola, A. F.

Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins

(Weistein, B, Ed.), Vol. 7, pp. 257-357, Marcel Dekker, New York (1983),

20   which describes the use of the methylenethio bioisostere [CH_2S] as an amide

replacement; and Szelke et al., In Peptides: Structure and Function,

Proceedings of the Eighth American Peptide Symposium, (Hruby and Rich,

Eds.); pp. 579-582, Pierce Chemical Co., Rockford, Ill. (1983), which

describes methyleneamino [CH_2NH] and hydroxyethylene [$CHOHCH_2$]

25   bioisosteres.

            The flexible portions of the structure can be replaced with suitable

bioisosteres or equivalents, so that the active conformation can be

maintained. As defined herein, the term "bioisostere" refers to atoms or

groups of atoms which are of similar size to the atom or group of atoms

30   which are to be replaced, wherein the compound containing the replacement

atom or group of atoms retains, to a substantial degree, the biological activity

of the original, unmodified peptide. See, for example, Nelson, Mautner, and

Kuntz, at pp. 227, 271 and 285, respectively, in <u>Burger's Medicinal</u>

<u>Chemistry</u>, Part 1, the Basis of Medicinal Chemistry, 4th Edition, M. E.

Wolff, ed. (John Wiley & Sons, NY, 1980).

Numerous peptide backbone substitutions are known to those of skill

5   in the art which can provide peptidomimetics with improved physical and

chemical properties, including enhanced rigidity and chemical and/or

metabolic stability. Suitable substitutions include modifying one or more of

the amide bonds by replacing the amide nitrogen with an oxygen atom, or a

sulfur atom, or by replacing H at the amide nitrogen with an alkyl, aryl,

10   aralkyl or alkaryl group, producing an N-substituted amide, or by replacing

the amide group with a methylene moiety, optionally substituted with one or

two alkyl, aryl, aralkyl or alkaryl groups, which can in turn optionally be

substituted with various functional groups, such as halogens, carbonyl

groups, amines, nitriles, azides, thiols, hydroxy groups, and carboxylic acid

15   groups. The alkyl groups are preferably C_{1-6} straight, branched or cyclic

groups. Further, one or more of the amide bonds present in the peptide

backbone can be modified, for example, by replacing the amide carbonyl

group with a methylene group (optionally substituted as described above), a

thiocarbonyl group, a sulfone moiety or a sulfoxide moiety.

20   The peptide can be further modified by introducing alkyl, aryl,

aralkyl or alkaryl substituents, optionally substituted as described above, at

one or more of the alpha-carbon atoms, such that the peptide backbone is

unchanged, but additional side chain substituents are present in the

chemically modified analog. Suitable α-carbon atom modifications include

25   cyclopropyl groups, ethylidene groups, and primary, secondary or tertiary

amines.

Each of these modifications can be introduced into the peptide chain

in either orientation (i.e., in the orientation shown, or in the "reverse"

orientation). In addition, various substituents on the amide nitrogen and the

30   α-carbon can be bound to one another, thereby forming a cyclic structure

which is a relatively constrained analog. Other constrained, cyclic structures

can also be prepared by linking various substituents to form cyclic structures

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using chemical techniques know. to those of skill in the art. Other

modifications include those described in U.S. Patent No. 5,612,895 to Balaji

et el., the contents of which are hereby incorporated by reference. .

Chemically modified analogs are typically more resistant to

5   enzymatic cleavage than the native peptides from which they are derived

because the modified residues are not typically recognized by the enzymes

which degrade naturally occurring proteins. Further, the backbone and side

chains of peptides can be modified to provide peptidomimetics with reduced

conformational flexibility. Accordingly, the possibility that the peptide will

10   adopt conformation(s) other than the specifically desired conformation(s) can

be substantially minimized by appropriate modification.

                    Methods of Chemically Preparing IgE Analogs

Once the desired analog (including backbone and side chain

modifications, as appropriate) has been identified, chemical synthesis is

15   undertaken, employing standard synthetic techniques. For a given target

compound, the skilled artisan can readily identify suitable synthetic

approaches for the preparation of the target compound. Particular techniques

for synthesizing certain classes of compounds are described in more detail

below.

20   Proteins can be expressed recombinantly or naturally and cleaved by

enzymatic digest, expressed from a sequence encoding just a peptide, or

synthesized using standard techniques. It is a routine matter to make

appropriate peptides, test for binding, and then utilize the peptides. The

peptides are easily prepared by standard techniques. They can also be

25   modified to increase *in vivo* half-life, by chemical modification of the amino

acids or by attachment to a carrier molecule or inert substrate, as discussed

above. The peptides can also be conjugated to a carrier protein by standard

procedures such as the commercial Imject kit from Pierce Chemicals or

expressed as a fusion protein, which may have increased stability. Solid

30   phase synthesis described by J. Merrifield, 1964 J. Am. Chem. Soc. 85,

2149, used in U.S. Patent No. 4,792,525, and described in U. S. Patent No.

4,244,946, wherein a protected alpha-amino acid is coupled to a suitable

resin, to initiate synthesis of a peptide starting from the C-terminus of the
peptide. Other methods of synthesis are described in U.S. Patent No.
4,305,872 and 4,316,891, the contents of which are hereby incorporated by
reference. These methods can be used to synthesize peptides having

5   identical sequence to the receptor proteins described herein, or substitutions
or additions of amino acids, which can be screened for activity as described
above.

The peptide can also be prepared as a pharmaceutically acceptable
acid- or base- addition salt, formed by reaction with inorganic acids such as

10   hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic
acid, sulfuric acid, and phosphoric acid, and organic acids such as formic
acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid,
oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by
reaction with an inorganic base such as sodium hydroxide, ammonium

15   hydroxide, potassium hydroxide, and organic bases such as mono-, di-,
trialkyl and aryl amines and substituted ethanolamines.

Peptides containing cyclopropyl amino acids, or amino acids
derivatized in a similar fashion, can also be used. These peptides retain their
original activity but have increased half-lives *in vivo*. Methods known for

20   modifying amino acids, and their use, are known to those skilled in the art,
for example, as described in U.S. Patent No. 4,629,784 to Stammer.

After the compounds are synthesized, their biological activity can be
evaluated, for example, using competitive binding studies, and iterative
refinement of the peptidomimetic (in the case of a constrained analog itself)

25   can then be carried out. Those chemically modified analogs which are
biologically active can be employed as peptidomimetics without further
modification.

II.   **Pharmaceutical Compositions**

Formulations

30   Dissociation of IgE from its receptor is extremely slow, exhibiting
half-times of days to weeks (Isersky, et al. J. Immunol. 122: 1926-1936,
1979). Consequently, IgE fragments bound to FcεRI should produce a stable

15

and long term block of these receptors' capacity to activate mast cells. It must also be noted, however, that in order to be effective, IgE fragments will need to occupy a sufficient number of receptor to block antigen-induced activation of the mast cells. Thus, any pharmaceutical preparation of IgE

5   fragments must be presented in sufficiently high concentration and for a sufficient length of time to displace native IgE from the patient's mast cell population.

The compounds described above are preferably administered in a pharmaceutically acceptable vehicle. Suitable pharmaceutical vehicles are

10   known to those skilled in the art. For parenteral administration, the compound will usually be dissolved or suspended in sterile water or saline. Carriers suitable for local release, including administration by inhalation or by injection into muscle for production of recombinant IgE by the individual to be treated, or topical administration, include ointments, salves, lotions,

15   gels, and controlled release formulations, such as liposomes or microspheres (or microparticles). Methods for preparing liposomes and microspheres for administration to a patient are known to those skilled in the art. U.S. Patent No. 4,789,734 describe methods for encapsulating biological materials in liposomes. Essentially, the material is dissolved in an aqueous solution, the

20   appropriate phospholipids and lipids added, along with surfactants if required, and the material dialyzed or sonicated, as necessary. A review of known methods is by G. Gregoriadis, Chapter 14. "Liposomes", Drug Carriers in Biology and Medicine pp. 287-341 (Academic Press, 1979). Microspheres formed of polymers or proteins are well known to those skilled

25   in the art, and can be tailored for passage through the gastrointestinal tract directly into the bloodstream. Alternatively, the compound can be incorporated and the microspheres, or composite of microspheres, implanted for slow release over a period of time, ranging from days to months. See, for example, U.S. Patent No. 4,906,474, 4,925,673, and 3,625,214.

30   The formulation and method for administration can be used to modulate the specific IgE responses. For example, as described by Vrtala, et al., Int. Arch. Allergy Immunol. 107(1-3), 290-294 (1995), an antigen can be

administered to an individual through the use of a recombinant expression host, such as an apathogenic Salmonella strain, which can be orally administered. Studies by Vrtala, et al., showed that this method induced a Th1 immune response with undetectable IgG1 or IgE.

5   Methods for Administration

One of the following four routes of administration to achieve the daily dosage calculated below for the required period of time.

A.   Direct subcutaneous injection.

B.   Implantation of a subcutaneous depot.

10   C.   Direct injection into a muscle of cDNA vector encoding the requisite portion of IgE fragments under the control of a strong muscle-specific promoter. Similar techniques have been employed to express factor VII in mice (Miller, et al. Gene Therapy 2: 736-742, 1995). To ensure that expression of the exogenous gene could be discontinued in the event that it

15   proved to be deleterious, IgE fragments coding sequence would be flanked by lox sequences. The sequence encoding the CRE recombinase would also be carried by the vector, under the control of a tight inducible promoter. Activation of the inducible promoter through ingestion of a small molecule (i.e., tetracycline) would activate the CRE recombinase, leading to the

20   excision of the lox-flanked IgE fragments sequence (Tsien, et al. Cell 87:1317-1326, 1996). Similarly, transfected MHC-matched cells expressing and secreting IgE fragments could be infused or implanted. Co-transfection of these cells with the cDNA encoding the Herpes Simplex Virus thymidine kinase would ensure that they could be killed through the administration of

25   acyclovir, should the need to eliminate them arise (Bonin, et al. Science 276: 1719-1724, 1997).

D.   Numerous studies demonstrate that the pulmonary epithelium is permeable to fairly large proteins (Gensch, et al. Science 157:1204-10206, 1967). The therapeutic potential of pulmonary administration of intact

30   proteins has been demonstrated for insulin (Elliot, et al. Aust. Paediatr. J. 23: 293-297, 1987) and is being explored for human growth hormone (Patton, et al. Biotech. Therap. 1:213-228, 1990). The serum levels attainable through

pulmonary administration are comparable to those which can be achieved

through parenteral administration (Patton, et al. Biotech. Therap. 1:213-228,

1990). It is likely, therefore, that sufficiently high plasma levels of IgE

fragments could be achieved through inhalation-based pulmonary

5   administration. It is also important to note that a very large proportion of

allergy symptoms are attributable to the degranulation of mast cells

embedded within the nasal and pulmonary epithelium. Furthermore, recent

evidence indicates that the IgE responsible for the nasal symptoms of allergy

is synthesized locally within the nose itself (Durham, et al. Eur. J. Immunol.

10   27: 2899-2906, 1997; Durham, et al. Int. Arch. of Allergy and Immunol.

113: 128-130, 1997). Inhalation might be expected to deliver extremely high

concentrations IgE fragments directly to this important population of nasal

and respiratory mast cells. Inhibition of allergen-induced degranulation of

nasal and pulmonary mast cells might be expected to dramatically ameliorate

15   symptoms such as allergic rhintis and bronchiolar constriction. Thus, even if

the circulating levels of IgE fragments which can be achieved by inhalation

are not sufficient to ensure that mast cells throughout the entire system are

disarmed, the local inactivation of pulmonary and nasal mast cells might be

sufficient to bring about significant symptomatic relief. It is likely,

20   therefore, that the dose of IgE fragment required to bring about relief of nasal

and respiratory allergic symptoms will be much smaller (and hence more

easily attainable and maintainable) than that required for the systemic dose

calculated below.

Dosages

25   In the preferred embodiment, pharmaceutically acceptable carriers

will typically by saline, phosphate buffered saline, or water, if the

composition is administered by injection. The pharmaceutical preparation of

the human IgE fragment, or analogue, is administered for the dual purposes

of occupying mast cell FcεRI receptors so as to prevent allergen-induced

30   degranulation and occupying FcεRII receptors to reduce circulating levels of

native IgE. This preparation serves as a pan-specific anti-allergy therapy,

relieving and preventing allergy symptoms independent of the nature of the

allergen. Consequently, patients allergic to multiple substances will be completely treated by this preparation, obviating the need for multiple courses of allergen-specific immunizations.

Previous animal studies indicate that systemic delivery of

5   approximately 25 mg/kg/day of monospecific IgE is sufficient to block subsequent passive sensitization with a different monospecific IgE (Spiegelberg, et al. J. Immunol. 136:131-135, 1986). However, this dose of IgE delivered daily over 13 days did not significantly diminish the allergic response in animals which had been actively immunized with a specific

10   allergen on day 0 or -3 of the protocol. Given the extremely long half-life of IgE bound to FcεRI at the mast cell surface (Isersky, et al. J. Immunol. 122: 1926-1936, 1979), this observation is not at all surprising. To attain therapeutic levels of displacement of native IgE from patient mast cells it will be necessary to maintain continuously high circulating levels of

15   exogenous IgE fragments for at least 6-8 weeks. Serum IgE concentrations in adults are approximately 10^{-8} g/ml (Nye, et al. Clin. Allergy 1:13-24, 1975). It is desirable, therefore, to maintain continuous serum IgE fragments concentrations of at least 5×10^{-6} g/ml. A similar 100-fold excess was sufficient to block the Prausnitz-Kustner passive immunication reaction in

20   human subjects (Geha, et al. Nature. 315:577-578, 1985). Since the half-life of circulating IgE is approximately 12 hours (Spiegelberg, et al. J. Immunol. 136:131-135, 1986), between approximately 0.4 and 0.8 G should constitute a reasonable upper estimate of the single daily dose required for a 70 kg individual.

25   **IV.   Assays for Efficacy**

Serum levels of IgE fragments can be measured by quantitative western blot analysis employing an [125I]-conjugated anti-IgE fragments antibody as a probe. Protein in serum samples is separated by SDS polyacrylamide gel electrophoresis (SDS-PAGE) followed by electropheretic

30   transfer to nitrocllulose paper. For quantitation purposes, a dilution series of known quantities of IgE is also to be loaded on separate lanes of the same gel. IgE fragments can be distinguished from native IgE by its distinctive

molecular weight. Labelled bands are excised and bound radioactivity
determined by γ-counting.

Fractional levels of IgE fragments bound to FcεRI can be determined
by quantitative western blotting. Peripheral blood basophils can be isolated
5 from patient serum (Weyer, et al. Clin. and Exp. All. 25:935-941, 1995) and
their associated proteins separated by SDS-PAGE followed by
electropheretic transfer. The relative quantity of native IgE versus IgE
fragments bound to the cells is determined using quantitative western blot
analysis employing an [^{125}I]-conjugated anti-IGE fragments antibody as a
10 probe. Native IgE is distinguished from IgE fragments by virtue of their
distinct molecular weights. Radioactivity in excised bands can be
quantitated by γ-counting and the native IgE/IgE fragments ratio determined.

The susceptibility of cells from treated patients to undergo cross-
linking dependent granule exocytosis can be determined with peripheral
15 blood basophils, prepared from patient serum (Weyer, et al. Clin. and Exp.
All. 25:935-941, 1995). Cells can be exposed to a bivalent IgG antibody
directed against the Fab portion of IgE. This reagent should not interact with
surface-bound IgE fragment. Degranulation is measured by standard
techniques (Weyer, et al. 1995). This treatment should not induce basophils
20 from successfully treated patients to degranulate. An IgG antibody directed
against the Fc portion of IgE is employed as a positive control to
demonstrate that the basophils from treated patients retain the capacity to
undergo cross-linking mediated degranulation.

We claim:

1.   A method of inhibiting an allergic response comprising administering to an individual in need thereof an effective amount to prevent clinically significant allergen-induced mast cell activation of a composition comprising

a pharmaceutically acceptable carrier and

a compound binding to an Fcε receptor to prevent occupancy of the receptor by native IgE, which cannot be cross-linked by antigen, and which does not elicit an immune reaction.

2.   The method of claim 1 wherein the compounds are selected from the group of compounds binding to the FcεRI on mast cells consisting of human IgE fragments, recombinant IgE fragments, and single chain IgE fragments.

3.   The method of claim 1 wherein the compounds are non-crosslinkable recombinant antibodies to FcεRI.

4.   The method of claim 1 wherein the compound is a peptidomimetic.

5.   The method of claim 1 wherein the compound encodes a molecule selected from the group of molecules binding to the FcεRI on mast cells consisting of IgE fragments, recombinant IgE fragments, single chain IgE fragments, and non-cross-linkable recombinant antibodies to FcεRI.

6.   The method of claim 1 wherein the composition is administered to the individual in need thereof by injection.

7.   The method of claim 1 wherein the composition is administered to the individual in need thereof by inhalation.

8.   The method of claim 1 wherein the composition is administered to the individual in need thereof locally or topically.

9.   The method of claim 1 formulated for controlled release.

10.   The method of claim 1 formulated to maximize mucosal adhesion and release.

11. A composition comprising

a pharmaceutically acceptable carrier and

an effective amount to prevent clinically significant allergen-induced mast cell activation of a composition comprising a compound binding to an Fcε receptor to prevent occupancy of the receptor by native IgE, which cannot be cross-linked by antigen, and which does not elicit an immune reaction.

12. The composition of claim 11 wherein the compounds are selected from the group of compounds binding to the FcεRI or FcεRII on mast cells consisting of IgE fragments, recombinant IgE fragments, and single chain IgE fragments.

13. The composition of claim 11 wherein the compounds are non-crosslinkable recombinant antibodies to FcεRI.

14. The composition of claim 11 wherein the compound is a peptidomimetic.

15. The composition of claim 11 wherein the compound encodes a molecule selected from the group of molecules binding to the FcεRI or FcεRII on mast cells consisting of IgE fragments, recombinant IgE fragments, single chain IgE fragments, and non-crosslinkable recombinant antibodies to FcεRI.

16. The composition formulated for controlled release.

17. The composition formulated to maximize mucosal adhesion.

18. The composition formulated for administration nasally or by inhalation.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   C07K   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 04807 A (GENENTECH) 13 February 1997 (1997-02-13) page 4, line 28 - line 39 page 9, line 12 - line 32 ---	1-18
X	EP 0 499 112 A (F. HOFFMANN-LA ROCHE) 19 August 1992 (1992-08-19) page 3, line 28 - page 4, line 10 page 5, line 28 - line 45 page 22- pae 23: claims 1-12 ---	1,3,5,6, 8,9,11, 13,15,16
X	WO 95 14779 A (RESEARCH EXPLOITATION LIMITED) 1 June 1995 (1995-06-01) page 4, line 19 - page 5, line 6 --- -/--	1,2,4,5, 11,12, 14,15

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not
     considered to be of particular relevance
"E" earlier document but published on or after the international
     filing date
"L" document which may throw doubts on priority claim(s) or
     which is cited to establish the publication date of another
     citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or
     other means
"P" document published prior to the international filing date but
     later than the priority date claimed

"T" later document published after the international filing date
     or priority date and not in conflict with the application but
     cited to understand the principle or theory underlying the
     invention
"X" document of particular relevance; the claimed invention
     cannot be considered novel or cannot be considered to
     involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention
     cannot be considered to involve an inventive step when the
     document is combined with one or more other such docu-
     ments, such combination being obvious to a person skilled
     in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 September 1999	13/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Flao, K

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 04173 A (GENENTECH) 4 March 1993 (1993-03-04) claims 15,16 ---	1,2,4-6, 11,12, 14,15
X	WO 96 12741 A (GLAXO GROUP LIMITED) 2 May 1996 (1996-05-02) page 11, line 10 - page 12, line 21 claims 1-4 -----	1,6-8, 10-12, 15,17,18

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:
   > Remark: Although claims 1-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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